Title

The bumble bee microbiome increases survival of bees exposed to selenate toxicity

Journal

Environmental Microbiology, 21(9)

ISSN

1462-2912 1462-2920

Authors

Rothman, Jason A
Leger, Laura
Graystock, Peter
et al.

Publication Date

2019-05-14

DOI

10.1111/1462-2920.14641

Peer reviewed

environmental microbiology

Environmental Microbiology (2019) **00**(00), 00–00 doi:10.1111/1462-2920.14641

The bumble bee microbiome increases survival of bees exposed to selenate toxicity

Jason A. Rothman,[1,2] Laura Leger,[2] Peter Graystock,[2,3] Kaleigh Russell[2] and Quinn S. McFrederick [1,2*]

[1]*Graduate Program in Microbiology, University of California, Riverside, CA, 92521, USA.*

[2]*Department of Entomology, University of California, Riverside, CA, 92521, USA.*

[3]*Department of Life Sciences, Imperial College London, Silwood Park Campus, Ascot, SL5 7PY, UK.*

Summary

Bumble bees are important and widespread insect pollinators who face many environmental challenges. For example, bees are exposed to the metalloid selenate when foraging on pollen and nectar from plants growing in contaminated soils. As it has been shown that the microbiome of animals reduces metalloid toxicity, we assayed the ability of the bee microbiome to increase survivorship against selenate challenge. We exposed uninoculated or microbiota-inoculated *Bombus impatiens* workers to a field-realistic dose of 0.75 mg l^{-1} selenate and found that microbiota-inoculated bees survive slightly but significantly longer than uninoculated bees. Using 16S rRNA gene sequencing, we found that selenate exposure altered gut microbial community composition and relative abundance of specific core bacteria. We also grew two core bumble bee microbes – *Snodgrassella alvi* and *Lactobacillus bombicola* – in selenate-spiked media and found that these bacteria grew in the tested concentrations of 0.001–10 mg l^{-1} selenate. Furthermore, the genomes of these microbes harbour genes involved in selenate detoxification. The bumble bee microbiome slightly increases survivorship when the host is exposed to selenate, but the specific mechanisms and colony-level benefits under natural settings require further study.

Received 12 September, 2018; revised 12 April, 2019; accepted 24 April, 2019. *For correspondence. E-mail quinnmc@ucr.edu; Tel. (+951) 827 5817; Fax (+951) 827-3086.

Introduction

Bumble bees (*Bombus* spp.) are important insect pollinators for a multitude of food crops and native plants (Klein *et al.*, 2007). Commercially, more than 1 million bumble bee colonies are used annually to pollinate high-value greenhouse crops such as tomatoes and peppers (Velthuis and van Doorn, 2006). Recently, it has been established that many wild bumble bee populations are steadily declining in North America (Cameron *et al.*, 2011) with some species having declined more than 90% (Colla *et al.*, 2012). European bumble bees are simultaneously facing serious decline (Goulson *et al.*, 2008). Much of these declines have been attributed to exposure to land use change, pesticides (Goulson *et al.*, 2015), parasites and pathogens (Graystock *et al.*, 2016), and heavy metals (Kosior *et al.*, 2007).

Selenium pollution is a worldwide problem stemming from industries such as mining, coal combustion and lubricant production as well as the leaching of selenium from seleniferous soils through rainfall or agricultural irrigation (Vickerman *et al.,* 2004). Plants growing in selenium-contaminated areas can accumulate high levels of the metalloid in their pollen and nectar which, once foraged upon by bees and other insect pollinators, can be toxic (Hladun *et al.,* 2011). For example, Quinn *et al.* (2011) found that bumble bees are not deterred from foraging on the selenium-accumulating plants *Brassica juncea* and *Stanleya pinnata* (flowers were found to contain up to 3200 mg kg^{-1} selenium) and that elevated levels of selenium accumulated both in their corbicular pollen and inside of the foraging bees (Quinn *et al.,* 2011). Likewise, Hladun *et al* found that honey bees (*Apis mellifera*) readily foraged on *Raphanus sativus* plants that were grown in seleniferous soil and collected pollen containing up to 2830 mg kg^{-1} selenium (Hladun *et al.,* 2012a). Accumulating selenium in the tissues of foraging honey bees has been shown to be detrimental to both the individual forager bees plus the health of the whole colony (Hladun *et al.,* 2012b; 2013; 2015). Metals may also have sub-lethal effects on bees, as foraging efficiency is decreased when honey bees are exposed to manganese (Sovik *et al.,* 2015) and bumble bees are exposed to nickel (Meindl and Ashman, 2013; 2014).

Selenium-tolerant microbes have been identified in a wide variety of environments, including bacteria isolated

from beetle larvae (Wang *et al.,* 2018), polluted water (Oremland *et al.,* 2004), mangrove soil (Mishra *et al.,* 2011), bioreactors (Soda *et al.,* 2011) and endophytic bacteria from hyperaccumulator plants (Sura-de Jong *et al.,* 2015). Additionally, some bacteria are known to reduce pernicious selenate and selenite ions to elemental selenium, thus significantly reducing toxicity (Lloyd, 2003), and facilitating removal from the bacterial cell (Debieux *et al.,* 2011). Not all bacteria, however, can tolerate metals and metalloids. For example, the microbiota of mice can be altered when exposed to lead, cadmium (Breton *et al.,* 2013) or selenium (Kasaikina *et al.,* 2011); human gut microbes change in response to arsenic and lead (Bisanz *et al.,* 2014); and the gut microbial community of the Mongolian toad is affected by a combination of copper, cadmium, zinc and lead (Zhang *et al.,* 2016). In light of these studies, using the microbiome to reduce metalloid toxicity is now starting to be explored, with Coryell *et al.* establishing that the mouse gut community assists in reducing host mortality upon arsenic exposure (Coryell *et al.,* 2018).

Previous research shows that the microbial associates of insects can detoxify some metals from the environment. Senderovich and Halpern found that the bacteria associated with the pollution-tolerant chironomid midges (Diptera: Chironomidae) detoxified lead and hexavalent chromium, which potentially reduces metals' harmful effects (Senderovich and Halpern, 2013). Likewise, Wang *et al.* showed that a strain of *Alcaligenes faecalis* isolated from beetle larvae (*Monochamus alternatus*) reduced selenite to the less toxic form of elemental selenium (Wang *et al.,* 2018). Still, there is little published research on the effects of selenium exposure on the microbiome of insects, despite the pervasive nature of selenium contamination in the environment (Lemly, 2004) and the importance of considering the microbiome of insects in entomological studies (Douglas, 2015). We seek to address this gap in the literature using bees, as in addition to being an emerging as a model for this type of research (Engel *et al.,* 2016), bees are frequently exposed to toxicants such as selenium when foraging, while the effects of environmental pollution on bumble bees and other wild bees are still an understudied field (Botías *et al.,* 2017).

Bumble bees are known to host a simple and distinct gut microbiome that comprises core bacterial species within the genera *Snodgrassella*, *Gilliamella*, *Lactobacillus*, *Bombiscardovia*, *Schmidhempelia* and *Bifidobacterium* (Martinson *et al.,* 2011; Koch and Schmid-Hempel, 2011a, b; Powell *et al.,* 2016; Kwong, Medina, *et al.,* 2017b). The honey bee and bumble bee microbiota is transmitted throughout the colony by social interactions between nest mates (Koch and Schmid-Hempel, 2011b) resulting in host-specific relationships within each clade of the corbiculate Apids (subfamily: Apinae) (Kwong *et al.,* 2017b). The microbiota has been shown to defend bumble bees against pathogens such as *Crithidia* spp. (Koch and Schmid-Hempel, 2011b; Palmer-Young *et al.,* 2018) and microbial dysbiosis of the core microbes has been suggested to encourage *Nosema* spp. establishment in honey bees (Maes *et al.,* 2016). Likewise, the honey bee microbiome is known to positively affect host health, by promoting weight gain (Zheng *et al.,* 2017), metabolizing toxic sugars (Zheng *et al.,* 2016), degrading pectin (Engel *et al.,* 2012) and stimulating immune function (Kwong *et al.,* 2017a).

Here, we investigate the complex interplay between selenate exposure and the bumble bee microbiome using an *in vitro*, *in vivo* and *in silico* methods. First, we ask: Does the *Bombus impatiens* microbiome increase survivorship against selenate toxicity? Second, are there effects of selenate exposure on the bees' microbial gut community and individual bacterial strains? Third, is there natural resistance against selenate exposure in the bumble bee core gut bacteria *Snodgrassella alvi* and *Lactobacillus bombicola in vitro*, and is there a possible genomic basis of bacterial selenate tolerance?

Materials and methods

Bee husbandry for the selenate-challenge experiment

We conducted two separate experiments to assay the effects of microbiome inoculation on selenate-challenged bees. For a pilot experiment (experiment 1, *see experimental design details*), we obtained three commercial *Bombus impatiens* colonies from the Biobest Group (Biobest USA, Romulus, MI), and for the fully factorial experiment (Experiment 2, *see experimental design details*), we obtained four commercial *B. impatiens* colonies from Koppert Biological Systems (Howell, MI). Each colony contained approximately 50 workers, a gravid queen, pollen and a proprietary sugar solution. As the supplied sugar solution typically contains antibiotics and antifungal compounds (Billiet *et al.,* 2016), we immediately replaced it with sterile 60% sucrose and allowed the bees access *ad libitum.* We also provided the colony with pollen patties *ad libitum* and kept the colonies in environmentally controlled rooms at 29°C under constant darkness at the University of California, Riverside. We allowed the colonies to grow undisturbed for 2 weeks before starting the experiment.

Uninoculated and microbiome-inoculated bumble bees

In order to manipulate the bumble bee gut microbiota, we used a modified version of previously described protocols (Koch and Schmid-Hempel, 2011b; Kwong *et al.,* 2014; Zheng *et al.,* 2017). We removed dark-coloured cocoons

from each of the three bee colonies and aseptically extracted the pupa from within each cocoon. We then placed pupae in a sterile, 48-well tissue culture plate (Corning, Corning, NY) and incubated the plates at 29°C with 70% humidity. Once the bees had eclosed, we placed them into cohorts of six colony mates in 475 ml polypropylene containers (WebstaurantStore, Lancaster, PA). We then autoclaved a mixture of 40% sucrose and pollen and provided the bees with this mixture *ad libitum* for 2 days.

To determine whether a healthy microbiota increases survivorship in bumble bees under selenate challenge, we fed selenate or a sterile sucrose control to bees that we either inoculated with gut microbes or left uninoculated. In order to inoculate bees with a stable microbial community, we fed cohorts of bees microbes harvested from workers corresponding to their source colony. To do this, we aseptically dissected the whole guts from three mature workers and macerated these in an autoclaved 40% sucrose and pollen solution. We then fed this homogenate *ad libitum* to the bees for 2 days, followed by sterile 40% sucrose for a total of 5 days to allow the microbes to stably colonize the gut (Powell *et al.,* 2014). The uninoculated bees did not receive microbes and were given a solution of sterile 40% sucrose and sterile pollen only.

Selenate exposure challenge and statistics

We conducted two separate experiments to determine whether the microbiota can increase bumble bee survival when challenged with field-realistic levels of selenate. In a preliminary experiment (exp. 1), we challenged $N = 87$ sham-inoculated bees and $N = 68$ microbiota-inoculated bees with 0.75 mg l^{-1} selenate (See the Supporting Information for details on exp. 1). This concentration was designed to represent exposure to a conservative concentration of selenate compared to what bees may naturally encounter when foraging upon plants in highly contaminated areas (up to 3200 mg kg^{-1}) (Quinn *et al.,* 2011) and from past greenhouse experiments (nectar up to 110 mg kg^{-1} and pollen 710 mg kg^{-1}) (Hladun *et al.,* 2011), as well as previously reported selenate toxicity to honey bees (Hladun *et al.,* 2013). As this preliminary experiment did not control for the effects of inoculation on bumble bee survival, we ran a second fully factorial experiment that crossed two factors: microbiota or sham inoculation and selenate spiked sucrose feed or sucrose only feed to control for the effects of a microbiome on control bees (exp. 2). We challenged 80 bees (40 microbe inoculated and 40 uninoculated) assigned to cohorts of four to seven colony mates with a solution of either 0.75 mg l^{-1} sodium selenate (Alfa Aesar, Ward Hill, MA) or 0 mg l^{-1} sodium selenate in 40% sucrose. In addition to the treatments, we also concurrently exposed 80 bees (43 microbe inoculated and 37 uninoculated) bees to 40% sucrose with no selenate to serve as controls. We allowed bees to feed *ad libitum* for up to 10 days, censused mortality daily and removed dead bees immediately upon discovery.

We analysed the mortality data using the Cox Proportional Hazards function with Mixed Effects (Therneau, 2015b) on colony of origin, microbe-inoculation and selenate treatment in R. We also checked to ensure that our data did not violate the proportional hazards assumptions of the Cox Regression with the function 'cox.zph' in the R package 'Survival,' (Therneau, 2015a) and graphed the survivorship data with the 'survminer' package in R (Kassambara and Kosinski, 2018). We used Schwartz's Bayesian information criterion (BIC) to choose the model that best fit our data and compensated for mixed effects through a penalized log likelihood.

Effects of sub-lethal doses of selenate on the bumble bee microbiome

To explore the effect of selenate on the bumble bee microbiome, we acquired three new bumble bee (*Bombus impatiens*) colonies containing <10 workers, a gravid queen, pollen and proprietary sugar solution (Koppert Biological Systems). We maintained the colonies in the same way as described earlier. We then isolated 20 individual mature workers from each colony ($N = 60$ total) in 60 ml polypropylene containers (WebstaurantStore) and provided them either 60% sucrose (control, $N = 30$) or 60% sucrose spiked with 0.5 mg l^{-1} sodium selenate (treatment, $N = 30$). Bees fed *ad libitum* for 4 days before we assessed mortality and stored the bees at −80°C. We used these bees for DNA extraction and 16S rRNA gene amplicon sequencing as described below.

DNA extraction and next-generation sequencing of the bacterial 16S rRNA gene

We used a modified DNA extraction protocol based on the study by Engel and colleagues (2013) and Pennington and and colleagues (2017b; 2018). Using sterile technique, we dissected whole guts out of each bee and placed them into 96-well bead-beating plates (Qiagen, Valencia, CA) containing 50–100 µl of 0.1 mm glass beads, one 3.4 mm steel-chrome bead (Biospec, Bartlesville, OK), and Qiagen lysis buffer, then homogenized the mixture with a Qiagen Tissuelyser at 30 Hz for 6 min. We included four blanks to control for contamination, which were included in all library preparation and sequence processing steps. We extracted total DNA from each sample with the DNeasy Blood and Tissue Kit (Qiagen) by following the manufacturer's protocol for tissue samples.

We prepared Illumina MiSeq libraries for paired-end sequencing as in the study by McFrederick and Rehan (2016), Pennington *et al.* (2017a) and Rothman *et al.*

(2018). We incorporated the genomic DNA primer sequence, an eight-mer barcode sequence, and Illumina adapter sequence as in (Kembel *et al.,* 2014). We used the primers 799F-mod3 (CMGGATTAGATACCCKGG) (Hanshew *et al.,* 2013) and 1115R (AGGGTTGCGCTCG TTG) (Kembel *et al.,* 2014) to amplify the V5-V6 region of the 16S rRNA gene. We used the following reaction conditions for PCR: 4 μl of DNA, 0.5 μl of 10 μM 799F-mod3 primer, 0.5 μl of 10 μM 1115R primer, 10 μl of sterile water and 10 μl 2× Pfusion High-Fidelity DNA polymerase (New England Biolabs, Ipswich, MA), an annealing temperature of 52°C, and 25 cycles in a C1000 Touch thermal cycler (BioRad, Hercules, CA). We then used the PureLink Pro 96 PCR Purification Kit (Invitrogen, Carlsbad, CA) to clean up the resulting amplicons. We subsequently performed a second PCR reaction using 1 μl of the cleaned PCR amplicons as a template with the primers PCR2F (CAA GCAGAAGACGGCATACGAGATCGGTCTCGGCATTCC TGC) and PCR2R (AATGATACGGCGACCACCGAGATC TACACTCTTTCCCTACACGACG) to generate the Illumina adapter sequence (Kembel *et al.,* 2014). We performed PCR with the following reaction conditions: 0.5 μl of 10 μM PCR2F primer, 0.5 μl of 10 μM PCR2R primer, 1 μl of cleaned PCR amplicon, 13 μl of sterile water and 10 μl of 2× Pfusion High-Fidelity DNA polymerase for 15 cycles at an annealing temperature of 58°C. We used 18 μl of the resulting amplicons for normalization with the SequalPrep Normalization kit and followed the supplied protocol (ThermoFisher Scientific, Waltham, MA). We pooled 5 μl of each of the normalized library and performed a final clean up with a PureLink PCR Purification Kit (Invitrogen). We then quality checked the amplicons on a 2100 Bioanalyzer (Agilent, Santa Clara, CA) and sequenced the libraries using a V3 Reagent Kit at 2 × 300 cycles on an Illumina MiSeq Sequencer (Illumina, San Diego, CA) in the UC Riverside Genomics Core Facility.

Raw sequencing data are available on the NCBI Sequence Read Archive (SRA) under accession numbers SRR6788889-SRR6788898, SRR6788969-SRR6788978, SRR6788989-SRR6789000 and SRR6789009-SRR6789022.

16S rRNA gene quantitative PCR for bacterial abundance in bees

We used quantitative PCR (qPCR) to validate our methods of rearing uninoculated or microbe-inoculated bees. We extracted DNA in the same way as above from individual control-treated bees that survived the full 10 days, as there was likely bacterial proliferation or degradation in bees that died during the experiment. We then ran 16S rRNA gene qPCR on the extracted DNA in triplicate using the following recipe: 2 μl DNA, 5 μl SsoAdvanced master mix (BioRad), 0.2 μl 10 μM forward primer (TCCTACGGGAGGCAGC AGT), 0.2 μl 10 μM reverse primer (GGACTACCAGGGTAT CTAATCCTGTT) (Nadkarni *et al.,* 2002), and 2.6 μl of sterile ultrapure water. We used a protocol consisting of an initial denaturation step of 95°C for 3 min, followed by 95°C for 10 s and an annealing/extension step of 59°C for 30 s repeated 39 times on a BioRad C1000 Touch thermal cycler. We compared our samples to a standard curve of 1×10^2 –1×10^8 copies of the 16S rRNA gene cloned into a TOPO-TA plasmid (Invitrogen), with all qPCR efficiencies between 90% and 100% and R^2 above 0.98 and tested our data for statistical significance using Welch's two-tailed t-test in R. Finally, we validated the DNA extractions by running PCR targeting a region of the bee 18S rRNA gene on each sample and verifying that there was a positive band on an agarose gel, as in the study by Meeus and colleagues (2009).

Bioinformatics and statistics

We used QIME2-2017.12 (Bolyen *et al.,* 2018) to process the 16S rRNA gene sequences. We viewed the sequence quality of our sequences and removed the low-quality ends. Then, we used DADA2 (Callahan *et al.,* 2016) to identify exact sequence variants (ESVs; 16S rRNA gene sequences that are identical), to remove chimeric sequences and to quality filter the data. We assigned taxonomy to the ESVs using the q2-feature-classifier (Bokulich *et al.,* 2018) with the SILVA 16S rRNA gene database (Quast *et al.,* 2013). We also conducted local BLASTn searches against the NCBI 16S ribosomal RNA sequences database (accessed March 2018). We then removed reads matching mitochondria and contaminants (Salter *et al.,* 2014) as identified in our blank samples from the feature tables. After filtering out contaminants, we aligned the representative sequences against the SILVA reference alignment with MAFFT (Katoh and Standley, 2013) and generated a phylogenetic tree using FastTree v2.1.3 (Price *et al.,* 2010). We used this tree and the filtered feature table to analyse alpha diversity, sampling depth, and to generate a Generalized UniFrac distance matrix (Chen *et al.,* 2012). We visualized the UniFrac distance through Principal Coordinates Analysis (PCoA), Non-metric Multidimensional Scaling (NMDS), and used R v3.4.1 (R Core Team, 2018) to plot the data. We analysed the alpha diversity of our samples through the Shannon Diversity Index and assessed statistical significance through the Kruskal–Wallis test in QIIME2. We also used the R packages 'vegan' (Oksanen *et al.,* 2017) to test for statistical significance through Adonis on the distance matrix, 'ggplot2' for graphing and 'DESeq2' to analyse differentially abundant ESVs representing at least 1% proportional abundance between treatments (Love *et al.,* 2014). To minimize the likelihood of a type I error due to differential data dispersion in our Adonis testing, we analysed the distance

matrix with PERMDISP (permutational dispersion of beta diversity with 999 permutations).

Bacterial culture conditions and inhibitory concentration analyses

To determine inhibitory concentrations of field-realistic doses of selenate on two representative members of the bumble bee core gut microbiota, we grew liquid cultures of *Snodgrassella alvi* wkB12 in Tryptic Soy Broth (TSB) (Becton, Dickinson, Franklin Lakes, NJ) and *Lactobacillus bombicola* DSM-28793 in De Man, Rogosa and Sharpe +0.05% cysteine (MRSC) broth (Research Products International, Mt. Prospect, IL). We incubated each culture at 37°C under a 5% CO_2 atmosphere inside a type C Biobag (Becton) with a CO_2 generation ampule and grew the cultures to an OD_{600} of 1.0. We then transferred 1 μl of the cultures to 199 μl of TSB or MRSC spiked with five concentrations of sodium selenate (0.001, 0.01, 0.1, 1.0 and 10 mg l^{-1}) in triplicate along with 0 mg l^{-1} selenate controls and media blanks under the same conditions as above. We allowed the cultures to grow for 48 h and read the cultures' OD_{600} with a VarioSkan Lux microplate reader (ThermoFisher Scientific). We then tested statistical significance with one-way ANOVA, Tukey's HSD post hoc testing, and normality through the Shapiro–Wilk test with the R package 'car' (Fox and Weisberg, 2011).

Genomic basis of resistance to selenate

We used the National Microbial Pathogen Data Resource's Rapid Annotations using Subsystem Technology (RAST) server to annotate the publicly available genomes of bacteria usually found within the bumble bee gut and other bacteria that were found to be differentially abundant between selenate exposure and controls (Overbeek *et al.,* 2005; Aziz *et al.,* 2008). We then searched through the genomes' subsystems for genes encoding selenium-containing proteins and functional genes corresponding to selenate reductases, selenocysteine acid metabolism, and the genes involved in uptake of selenate/selenite. To verify the accuracy of the RAST annotations, we also searched for protein homology using Swiss-Prot (Bateman *et al.,* 2017) and considered proteins with greater than 50% amino acid sequence identity to be homologous. Accession numbers for each representative strains' assembled genome are as follows: *Bifidobacterium bohemicum* DSM-22767 (GCA_000741525.1), *Bifidobacterium bombi* DSM-19703 (GCA_000737845.1), *Bifidobacterium commune* R-52791 (GCA_900094885.1), *Bombiscardovia coagulans* DSM-22924 (GCA_002259585.1), *Commensalibacter intestini* A911 (GCA_000231445.2), *Gilliamella apicola* wkB30 (GCA_000695585.1), *Gilliamella bombi* LMG-29879 (GCA_900103255.1), *Gilliamella intestini* R-53144 (GCA_900094935.1), *Lactobacillus apis* Hma11 (GCA_000970735.1), *Lactobacillus bombicola* R-53102 (GCA_900112665.1), *Lactobacillus mellis* Hon2 (GCA_000967245.1), *Candidatus Schmidhempelia bombi* Bimp (GCA_000471645.1), *Serratia marcescens* WW4 (GCA_000336425.1), and *Snodgrassella alvi* wkB12 (GCA_000695565.1). RAST annotation tables can be found in the Supporting Information File SF1.

Results

The bumble bee microbiome increases survival against selenate toxicity

In both the preliminary experiment (exp. 1) and the fully factorial experiment (exp. 2), the inoculated bumble bee microbiome significantly increased bee survival when exposed to selenate. In the preliminary experiment (exp. 1), the inoculated microbiome significantly increased bee survival (N = 155, Z = −3.27, P = 0.001); (Cox mixed-effects model fitted with penalized log-likelihood: χ^2 = 54.34, d.f. = 2.7, P < 0.001, BIC = 40.70), with microbiome-inoculated bees experiencing a 42% increase in mean survival (Fig. S1), although no bees lived the full 10 days. This result was replicated in Exp. 2 (N = 160, Z = −3.12, P = 0.002); (Cox mixed-effects model fitted with penalized log-likelihood: χ^2 = 88.81, d.f. = 3.6, P < 0.001, BIC = 73.00) with proper controls. We continued Exp. 2 for 10 days and found that selenate-challenged bees inoculated with a microbiome experienced a 20% increase in mean survival (Fig. 1). In the absence of selenate exposure, microbe inoculation did not significantly affect mortality when compared to uninoculated bees (N = 80, Z = −0.57, P = 0.57). Our selenate exposure data did not violate the assumptions of the Cox Proportional Hazards Model by inoculation treatment (ρ = −0.09, χ^2 = 0.63, P = 0.43), colony of origin (ρ = −0.05, χ^2 = 0.26, P = 0.68), selenate treatment (ρ = 0.19, χ^2 = 3.61, P = 0.06) or globally (χ^2 = 4.53, P = 0.48).

Finally, we verified that the uninoculated bees had depauperate microbiota compared to inoculated bees in our fully factorial experiment (exp. 2) through qPCR targeting the bacterial 16S rRNA gene on our control samples as in the study by Powell and and colleagues (2014) and Kesnerova and and colleagues (2017). Inoculated bees had a mean 16S rRNA gene copy number of 6.88×10^8 versus 8.89×10^4 for uninoculated bees (Welch's two-tailed t-test, t = 3.13, P = 0.004; Supporting Information Fig. S2).

Sub-lethal selenate exposure alters the microbiome of bumble bees

Alpha diversity and library coverage. There was a total of 276 126 quality-filtered reads with an average of 5210 reads per sample (N = 53) that were clustered into

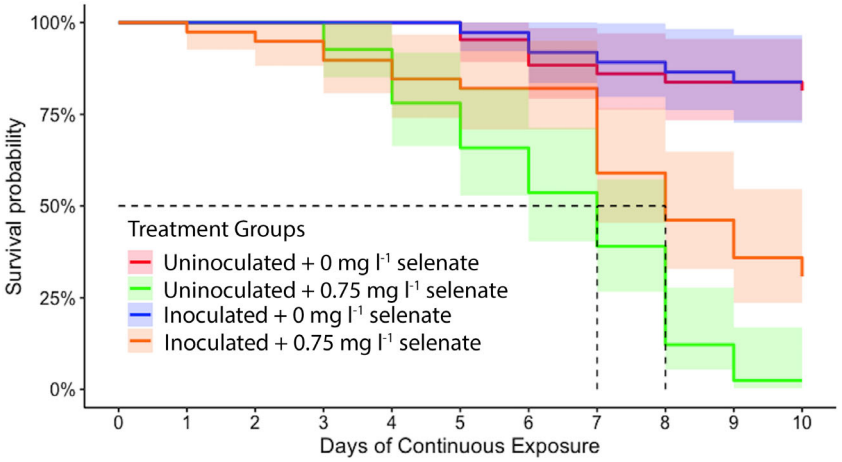

Fig. 1. Survival plot of the fully factorial experiment 2. Microbiome-inoculated bees lived significantly longer than uninoculated bees when challenge with 0.75 mg l^{-1} sodium selenate (N = 160, Z = –3.12, P = 0.002). Microbe inoculation did not affect mortality when compared to uninoculated bees in our controls (N = 80, Z = –0.57, P = 0.57). Shaded areas signify 95% confidence intervals, and dashed lines indicate 50% survival probability.

86 filtered exact sequence variants (ESVs). Through rarefaction analysis, we determined that we had representative coverage of bacterial species diversity at a depth of 2385 reads per sample (Supporting Information Fig. S3). We found that there was a significant increase in the alpha diversity (as measured by the Shannon Diversity Index) of the bees' microbial community when treated with sodium selenate (H = 7.95, P = 0.005).

Beta diversity and differential abundance of bacterial taxa

In order to discern patterns in the beta diversity of the whole microbial gut community of the bumble bees, we plotted the relative proportional abundance of ESVs comprising at least 1% of each sample (Fig. 2). Overall, we found our samples were dominated by the genera *Snodgrassella*, *Gilliamella*, *Lactobacillus*, *Bifidobacterium*, *Commensalibacter*, *Bombiscardovia* and *Serratia.* We also performed PCoA and NMDS analysis on the Generalized UniFrac distance matrix that compared selenate-treated bees with controls (Fig. 3). Overall, there was no obvious clustering by treatment in both the two-dimensional NMDS (stress = 0.18) and PCoA ordinations. As we performed the experiment on individual bees from three separate colonies, we then analysed the UniFrac distance matrix with Adonis (PERMANOVA with 999 permutations) using both treatment and colony of origin as explanatory variables in the model. We found that there was a significant effect of selenate treatment (F = 2.9, R^2 = 0.05, P < 0.001), colony (F = 3.30, R^2 = 0.12, P < 0.001) and interaction between treatment and colony (F = 1.87, R^2 = 0.07, P = 0.005) after 4 days of continuous exposure, and that our data were not heterogeneously dispersed (F = 0.89, P = 0.35). Our analyses found that while there are significant effects of treatment and colony, the small R^2 indicates that the impact of treatment on the overall beta diversity is slight, and the more physiologically important effects of selenate exposure are likely to be found at the individual ESV level.

We analysed the 16S amplicon data with 'DESeq2' to identify ESV changes within the bumble bee microbiome. Through analysing ESVs present at greater than 1% proportional abundance, we found nine differentially abundant ESVs (Benjamini and Hochberg corrected for multiple comparisons P_{adj} < 0.05. ESVs can be found in the Supporting Information SF2) between selenate-treated bees and controls in the following genera: 2 ESVs of *Commensalibacter intestini*, 3 ESVs of *Gilliamella apicola*, 2 ESVs of *Lactobacillus bombicola* and 2 ESVs of *Snodgrassella alvi.* Each of the ESVs was less proportionally abundant in selenate-treated bees except *C. intestini* (Fig. 4).

Genes involved in selenium ion uptake and processing

By using RAST subsystem analyses and UniProt BLAST searches, we identified the presence or the absence of genes that encode the production of selenium-containing proteins, selenate reductases, selenocysteine metabolism and genes involved in selenium ion uptake/release (Supporting Information Table ST1). We found that *G. apicola* wkB30 and *S. alvi* wkB12 only had one putative selenocysteine-containing enzyme each (both had formate dehydrogenase EC 1.17.1.9 based on sequence homology), while no other bacteria commonly found in bumble bee guts or our samples contained any selenoproteins. Many of the non-bumble bee-specific taxa had at least some active selenoproteins (Supporting Information Table ST1). Each of the other core bumble bee gut bacteria or non-core bacteria that were differentially abundant in our study had putative genes corresponding to the sulfate and thiosulfate import ATP-binding protein CysA (Lindblow-Kull *et al.,* 1985), the putative (Guzzo and Dubow, 2000) selenite-inducible transporter TsgA (Guzzo and Dubow, 2000), the selenate/selenite transporter DedA (Ledgham *et al.,* 2005), L-seryl-tRNA(Sec) selenium transferase SelA

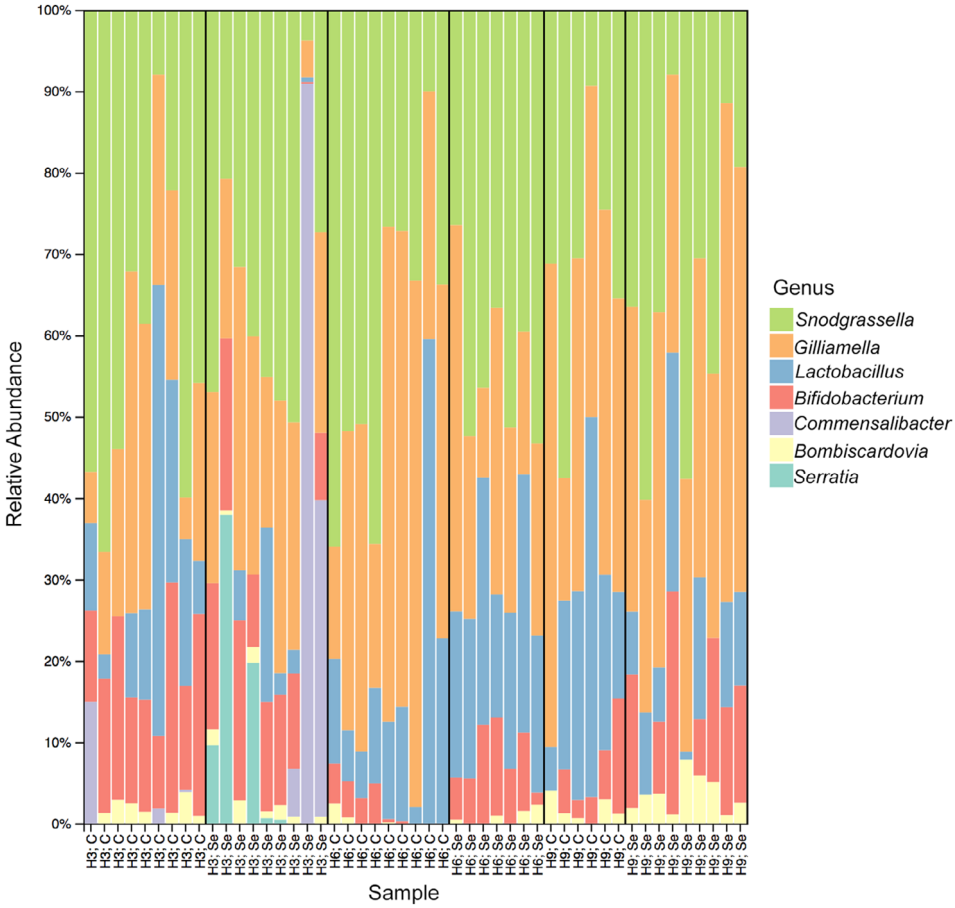

Fig. 2. Stacked bar plot showing the relative proportion of bacterial genera that were present at greater than 0.1% abundance in each sample. Individual sample treatments are indicated by 'C' for control and 'Se' for selenate exposure, and colony of origin is denoted by H3, H6 or H9.

(Forchhammer and Bock, 1991), the selenocysteine-specific translation elongation factor SelB (Rother *et al.,* 2000), the selenide/water dikinase SelD (Veres *et al.,* 1992) and the selenophosphate-dependent tRNA 2-selenouridine synthase 2-SeU (Veres and Stadtman, 1994). Only Candidatus *Schmidhempelia bombi* had no enzymes for the uptake or release of selenium ions or selenoprotein metabolism.

Members of the bumble bee microbiota react differently to selenate exposure

Through ANOVA testing, we did not detect an overall significant difference in bacterial growth after 48 h for either *Snodgrassella alvi* wkB12 ($F_{(5,12)}$ = 2.389, P = 0.101) or *Lactobacillus bombicola* ($F_{(5,12)}$ = 0.282, P = 0.914), at any dose of sodium selenate (Tukey's HSD P_{adj} > 0.05 for each concentration) (Fig. 5). Our data did not violate

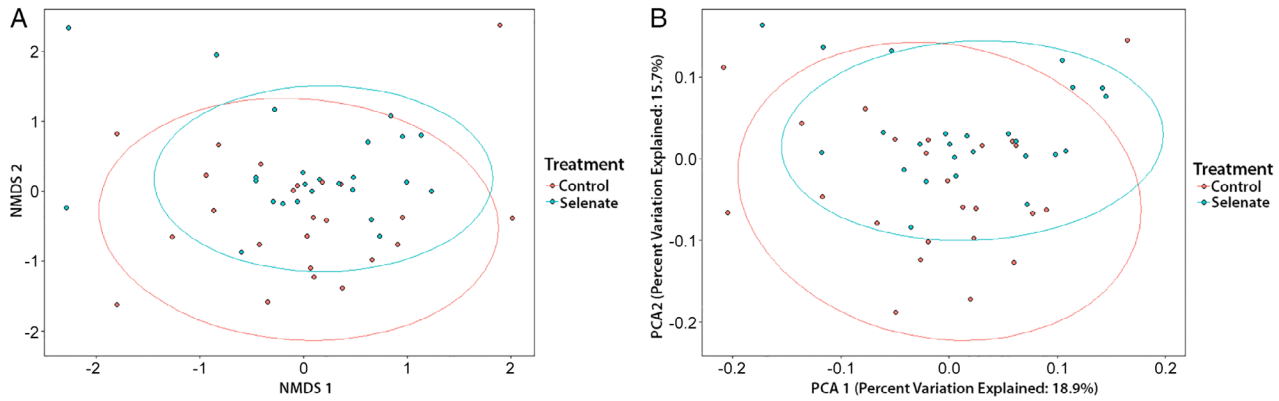

Fig. 3. (A) Nonmetric Multidimensional Scaling (stress = 0.18). (B) Principal Coordinates Analysis plot of the Generalized UniFrac distance matrices of individual bumble bee worker guts when exposed to sodium selenate versus controls. Red points indicate control treatments and blue points denote selenate treatments. Coloured ellipses designate 95% confidence intervals around the centroid median of the points.

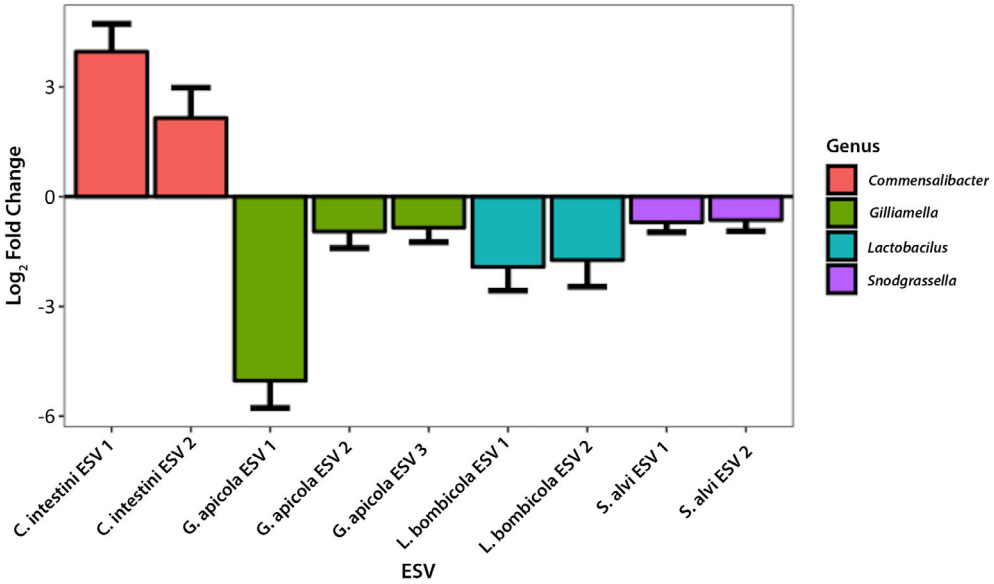

Fig. 4. Log_2fold change of the proportionally differentially abundant Exact Sequence Variants as measured by DESeq2 between selenate-treated bees and controls, coloured by genus. We analysed ESVs that were present in at least 1% proportional abundance, and each of the illustrated ESVs were found to be significantly different ($P_{adj} < 0.05$). See Supporting Information SF2 for feature ID of each taxa. Error bars denote the standard error of the Log_2fold change.

the assumption of normality, based on the Shapiro–Wilk Test (P = 0.892 and 0.613 respectively). We note that these bacteria grew somewhat poorly, indicating that our culturing conditions are not optimal for bee symbionts. Similar OD readings, however, have been reported in other studies using different strains of these bacteria (Raymann *et al.,* 2018).

Discussion

The *Bombus impatiens* microbiome plays a role in the reduction of host mortality when bees are exposed to field-realistic doses of selenium. We found that selenate toxicity was slightly higher in uninoculated bees than in those that we inoculated with a microbial community obtained from their colony mates and that lacking a microbiome did not affect survivorship of bees receiving only control treatments. To the best of our knowledge, this is the first time that the gut microbiome of any insect has been shown to increase the survivorship of its host against selenate poisoning. We show that while ingestion of selenate inflicts mortality on all tested bumble bees, the microbiome-inoculated bees have slightly reduced mortality, which on a wider colony level, may have a positive effect on resource-gathering, colony hygiene, and ultimately overall colony health. Future research into the colony-level effects of selenate poisoning on bumble bees should be investigated in a similar manner to the study by Hladun and and colleagues (2015), who showed that selenate had a deleterious effect on honey bee colony health.

Other work has linked the insect microbiota to metal/metalloid detoxification. Senderovich and Halpern (2013)

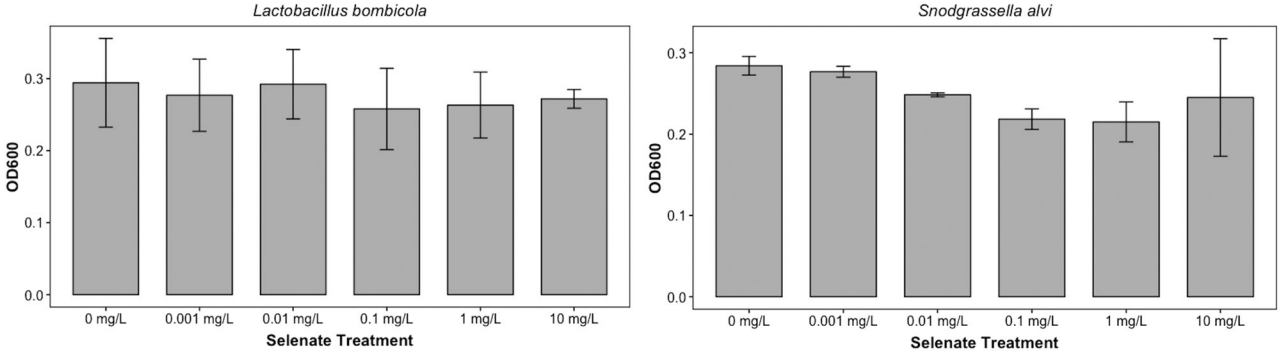

Fig. 5. Bar plots of the effects of sodium selenate exposure on *Snodgrassella alvi* and *Lactobacillus bombicola* growth after 48 h. Growth was not significantly affected for either *S. alvi* ($F_{(5,12)}$ = 2.389, P = 0.101) or *L. bombicola* ($F_{(5,12)}$ = 0.282, P = 0.914) at any concentration. Error bars denote standard error.

showed that bacteria associated with *Chironomus transvaalensis* egg masses and larvae reduced lead- and hexavalent chromium-induced mortality. Our research extends this work, as *Chironomus* spp. typically exhibit a lifestyle whereby adults do not feed (Pinder, 1986), do not possess a functional microbiome and are unlikely to orally ingest pollutants. Likewise, Wang *et al.* recently showed that a strain of bacteria isolated from beetle larvae can reduce selenite (Wang *et al.,* 2018), although as beetles undergo complete metamorphosis, this bacteria is unlikely to persist into the adult stage. These are important distinctions, as our study shows that the microbiome of adult bumble bees increases survivorship upon metalloid challenge during their final life stage. Furthermore, socially acquired core bumble bee gut bacteria appear to mainly drive the increase in survivorship. As pupal bees shed their larval gut and lose any alimentary tract bacteria upon eclosion (Koch and Schmid-Hempel, 2011b), any microbially mediated effect on larvae may be lost before the adult bees are exposed to the toxicant. Additionally, as we are studying coevolved, socially transmitted core microbes, these symbiotic bacteria share an intricate relationship with their host that includes defensive functions (Koch and Schmid-Hempel, 2011b). Our system serves as a useful model for studying transmittable symbiont-induced phenotypes that increase survival against environmental toxicants in social insects. Finally, it has been shown that the gut microbiota is involved in reducing arsenic-induced mouse mortality (Coryell *et al.,* 2018), and our research contributes to the growing body of literature that implicates the animal microbiome in increased host survival when challenged with metalloids.

We found ESVs of the gut symbionts *S. alvi*, *G. apicola* and *L. bombicola* in lower proportional abundance in selenate-treated bees versus controls. We also found two *C. intestini* ESVs in higher proportional abundance of in selenate-treated bees. This contrasts with our culture-based results in which *S. alvi* and *L. bombicola* were not affected by field-realistic, low doses of selenate. This conflicting result may be due to the compositional nature of microbiome data (Gloor *et al.,* 2017) in which other changes in proportional abundance may not reflect changes in absolute abundance. Likewise, these bacteria grew poorly in culture, which may mask the true effects of dose-dependent selenate exposure as an artefact, although strains of *S. alvi* and other Firm-5 lactobacilli have grown to similar OD_{600} readings in the previous studies (Raymann *et al.,* 2018). We also cannot examine genomic differences between cultured strains and taxa identified in our bee samples as we are unable to discern their entire genomes from a 16S rRNA gene sequencing survey. Strain level diversity in the honey bee gut microbiota is high (Engel *et al.,* 2012), and future experiments are needed to fully understand selenotolerance in the bumble bee microbiota. While there is no published research on the interactions of bee symbionts and selenium, non-bee-associated bacteria are known to accumulate selenium in culture (Calomme, Van den Branden, and Vanden Berghe 1995), or can respire less toxic elemental selenium (Lloyd, 2003; Debieux *et al.,* 2011; Wang *et al.,* 2018). This may be a mechanism for increasing host survivorship upon selenate challenge, and future studies should investigate the ability of symbionts to accumulate or respire selenium.

There are interesting patterns that develop when examining the genomes of bacterial genera typically associated with bumble bees: *Bifidobacterium* spp., *Bombiscardovia coagulans* and *Lactobacillus* spp. appear only to uptake selenium ions via the transporter DedA. *Snodgrassella alvi* wkB12 possesses DedA along with CysA, which is involved in selenium ion transport and can incorporate selenocysteine into proteins, which may contribute to selenate resistance. Candidatus *S. bombi* and *G. apicola* wkB30 also use selenocysteine but lack any obvious method of selenate uptake; although *G. apicola* genomes may vary between strains (Zheng *et al.,* 2016) and more investigation into their selenium metabolism is needed. While most differentially abundant bacteria were less proportionally abundant in the selenate treatments, one taxon was notably more abundant: *Commensalibacter.* The effects of selenium on this genus are unknown, although it does not possess DedA, but appears selenotolerant *in vivo.* Notably, we only obtained ESVs of *Commensalibacter* and the opportunistic bee pathogen *Serratia* from one colony, indicating this colony may be suffering from dysbiosis or disease and the apparent selenotolerance may be due of the compositional nature of our data.

Selenoproteins are common throughout several insects species, and their genomes often contain enzymes for selenium metabolism (Chapple and Guigó, 2008). Conversely, bumble bees and other hymenopterans are not known to incorporate selenium into proteins (Sadd *et al.,* 2015). However, the mechanisms of increased bee survival may be host mediated, as gut microbes could induce changes in host gene expression to generally allow for detoxification. Likewise, selenate-induced stress may synergize with the lack of a microbiome in our uninoculated bees, and we may be observing the combination of multiple insults on bee health. This may decrease host survivorship rather than the microbiome itself increasing survivorship, and more research needs to be conducted to understand the mechanisms of multiple stressors on bees. Microbial inducement of the immune system has been shown in honey bees (Kwong *et al.,* 2017a) and stimulation of detoxification gene expression and immune function has been shown to occur in bumble bees (Näpflin and Schmid-Hempel, 2016), so the presence

of the bees' microbiota may be influencing the bees ability to detoxify selenate. Finally, the bacteria may simply be forming a physical barrier, but more research is needed to test these competing hypotheses.

Our results illustrate that the bumble bee microbiome slightly increases host survival when exposed to selenate and that bacteria within the core microbiome are tolerant to field-realistic doses of selenate. Selenate causes shifts in the relative abundance of core microbes at the individual ESV level. As the mechanisms of the microbiome-induced increase in host survival upon selenate challenge are unknown, future research should investigate the ability of bacterial symbionts to metabolize and detoxify selenate in the host. Similarly, many bee species commonly encounter metal and metalloid contamination in the environment (Kosior *et al.,* 2007) and more studies are needed to assess the effects of other toxicants on their microbes.

Conflict of Interest

The authors declare that we have no competing interests.

Authors' contributions

JAR, LL, PG, KAR and QSM participated in designing and running the experiments and drafting of the manuscript. JAR carried out the statistical analyses and bioinformatics. All authors gave final approval for publication.

Acknowledgements

The authors would like to thank the UC Riverside Genomics Core facility staff for their Next-Generation Sequencing expertise. We are also grateful to Eli Powell, Waldan Kwong, and Nancy Moran for supplying bacterial strains. This research was supported by Initial Complement funds and NIFA Hatch funds (CA-R-ENT-5109-H) from UC Riverside to Quinn McFrederick and through fellowships awarded to Jason A. Rothman by the National Aeronautics and Space Administration MIRO Fellowships in Extremely Large Data Sets (Award No: NNX15AP99A) and the United States Department of Agriculture National Institute of Food and Agriculture Predoctoral Fellowship (Award No. 2018-67011-28123).

References

Aziz, R.K., Bartels, D., Best, A.A., DeJongh, M., Disz, T., Edwards, R.A., *et al.* (2008) The RAST server: rapid annotations using subsystems technology. *BMC Genomics* **9**: 75.

Bateman, A., Martin, M.J., O'Donovan, C., Magrane, M., Alpi, E., Antunes, R., *et al.* (2017) UniProt: the universal protein knowledgebase. *Nucleic Acids Res.* **45**: D158–D169.

Billiet, A., Meeus, I., Van Nieuwerburgh, F., Deforce, D., Wäckers, F., and Smagghe, G. (2016) Impact of sugar syrup and pollen diet on the bacterial diversity in the gut of indoor-reared bumblebees (*Bombus terrestris*). *Apidologie* **47**: 548–560.

Bisanz, J.E., Enos, M.K., Mwanga, J.R., Changalucha, J., Burton, J.P., Gloor, G.B., and Reid, G. (2014) Randomized open-label pilot study of the influence of probiotics and the gut microbiome on toxic metal levels in Tanzanian pregnant women and school children. *mBio* **5**: e01580–e01514.

Bokulich, N.A., Kaehler, B.D., Rideout, J.R., Dillon, M., Bolyen, E., Knight, R., *et al.* (2018) Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. *Microbiome* **6**: 90.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C., Al Ghalith, G.A., *et al.* (2018) QIIME 2: reproducible, interactive, scalable, and extensible microbiome data science. *PeerJ* **9**: 10.

Botías, C., David, A., Hill, E.M., and Goulson, D. (2017) Quantifying exposure of wild bumblebees to mixtures of agrochemicals in agricultural and urban landscapes. *Environ Pollut.* **222**: 73–82.

Breton, J., Massart, S., Vandamme, P., De Brandt, E., Pot, B., and Foligné, B. (2013) Ecotoxicology inside the gut: impact of heavy metals on the mouse microbiome. *BMC Pharmacol. Toxicol.* **14**: 62.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., and Holmes, S.P. (2016) DADA2: high-resolution sample inference from Illumina amplicon data. *Nat Methods* **13**: 581–583.

Calomme, M.R., Van den Branden, K., and Vanden Berghe, D.A. (1995) Selenium and Lactobacillus species. *J. Appl. Bacteriol.* **79**: 331–340. https://doi.org/10.1111/j.1365-2672.1995.tb03145.x.

Cameron, S.A., Lozier, J.D., Strange, J.P., Koch, J.B., Cordes, N., Solter, L.F., and Griswold, T.L. (2011) Patterns of widespread decline in north American bumble bees. *Proc Natl Acad Sci U S A* **108**: 662–667.

Chapple, C.E., and Guigó, R. (2008) Relaxation of selective constraints causes independent selenoprotein extinction in insect genomes. *PLoS One* **3**: e2968.

Chen, J., Bittinger, K., Charlson, E.S., Hoffmann, C., Lewis, J. D., Wu, G.D., *et al.* (2012) Associating microbiome composition with environmental covariates using generalized UniFrac distances. *Bioinformatics* **28**: 2106–2113.

Colla, S.R., Gadallah, F., Richardson, L.L., Wagner, D., and Gall, L. (2012) Assessing declines of north American bumble bees (Bombus spp.) using museum specimens. *Biodivers Conserv* **21**: 3585–3595.

Coryell, M., McAlpine, M., Pinkham, N.V., McDermott, T.R., and Walk, S.T. (2018) The gut microbiome is required for full protection against acute arsenic toxicity in mouse models. *Nat Commun.* **9**: 5424.

Debieux, C.M., Dridge, E.J., Mueller, C.M., Splatt, P., Paszkiewicz, K., Knight, I., *et al.* (2011) A bacterial process for selenium nanosphere assembly. *Proc Natl Acad Sci U S A.* **108**: 13480–13485.

Douglas, A.E. (2015) Multiorganismal insects: diversity and function of resident microorganisms. *Annu Rev Entomol* **60**: 17–34.

Engel, P., James, R.R., Koga, R., Kwong, W.K., McFrederick, Q.S., and Moran, N.A. (2013) Standard methods for research on *Apis mellifera* gut symbionts. *J Apic Res* **52**: 1–24.

Engel, P., Kwong, W.K., McFrederick, Q.S., Anderson, K.E., Barribeau, S.M., Chandler, J.A., *et al.* (2016) The bee microbiome: impact on bee health and model for evolution and ecology of host–microbe interactions. *mBio* **7**: e02164–e02115.

Engel, P., Martinson, V.G., and Moran, N.A. (2012) Functional diversity within the simple gut microbiota of the honey bee. *Proc Natl Acad Sci U S A* **109**: 11002–11,007.

Forchhammer, K., and Bock, A. (1991) Selenocysteine synthase from *Escherichia coli*. *J Biol Chem.* **266**: 6324–6328.

Fox, J., and Weisberg, S. (2011) *An {R} Companion to Applied Regression*, 2nd ed. Thousand Oaks, CA: SAGE Publications, Inc.

Gloor, G.B., Macklaim, J.M., Pawlowsky-Glahn, V., and Egozcue, J.J. (2017) Microbiome datasets are compositional: and this is not optional. *Front Microbiol.* **8**: 2224.

Goulson, D., Lye, G.C., and Darvill, B. (2008) Decline and conservation of bumble bees. *Annu Rev Entomol.* **53**: 191–208.

Goulson, D., Nicholls, E., Botias, C., and Rotheray, E.L. (2015) Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science* **347**: 1255957–1255957.

Graystock, P., Blane, E.J., McFrederick, Q.S., Goulson, D., and Hughes, W.O.H. (2016) Do managed bees drive parasite spread and emergence in wild bees? *Int J Parasitol Parasites Wildlife* **5**: 64–75.

Guzzo, J., and Dubow, M.S. (2000) A novel selenite- and tellurite-inducible gene in *Escherichia coli*. *Appl Environ Microbiol.* **66**: 4972–4978.

Hanshew, A.S., Mason, C.J., Raffa, K.F., and Currie, C.R. (2013) Minimization of chloroplast contamination in 16S rRNA gene pyrosequencing of insect herbivore bacterial communities. *J Microbiol Methods* **95**: 149–155.

Hladun, K.R., Di, N., Liu, T.-X.X., and Trumble, J.T. (2015) Metal contaminant accumulation in the hive: consequences for whole-colony health and brood production in the honey bee (*Apis mellifera* L.). *Environ Toxicol Chem* **35**: 322–329.

Hladun, K.R., Kaftanoglu, O., Parker, D.R., Tran, K.D., and Trumble, J.T. (2013) Effects of selenium on development, survival, and accumulation in the honeybee (*Apis mellifera* L.). *Environ Toxicol Chem.* **32**: 2584–2592.

Hladun, K.R., Parker, D.R., Tran, K.D., and Trumble, J.T. (2012a) Effects of selenium accumulation on phytotoxicity, herbivory, and pollination ecology in radish (*Raphanus sativus* L.). *Environ Pollut.* **172**: 70–75.

Hladun, K.R., Parker, D.R., and Trumble, J.T. (2011) Selenium accumulation in the floral tissues of two Brassicaceae species and its impact on floral traits and plant performance. *Environ Exp Bot* **74**: 90–97.

Hladun, K.R., Smith, B.H., Mustard, J.A., Morton, R.R., and Trumble, J.T. (2012b) Selenium toxicity to honey bee (*Apis mellifera* L.) pollinators: effects on behaviors and survival. *PLoS One* **7**: e34137.

Kasaikina, M.V., Kravtsova, M.A., Lee, B.C., Seravalli, J., Peterson, D.A., Walter, J., *et al.* (2011) Dietary selenium affects host selenoproteome expression by influencing the gut microbiota. *FASEB J.* **25**: 2492–2499.

Kassambara, A. and Kosinski, M. (2018) *Survminer: Drawing Survival Curves Using 'ggplot2*.' https://CRAN.R-project.org/package=survminer.

Katoh, K., and Standley, D.M. (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol.* **30**: 772–780.

Kembel, S.W., O'Connor, T.K., Arnold, H.K., Hubbell, S.P., Wright, S.J., and Green, J.L. (2014) Relationships between phyllosphere bacterial communities and plant functional traits in a neotropical forest. *Proc Natl Acad Sci USA* **111**: 13715–13,720.

Kesnerova, L., Mars, R.A.T., Ellegaard, K.M., Troilo, M., Sauer, U., and Engel, P. (2017) Disentangling metabolic functions of bacteria in the honey bee gut. *PLOS Biol.* **15**: e2003467.

Klein, A.-M., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., and Tscharntke, T. (2007) Importance of pollinators in changing landscapes for world crops. *Proc Biol Sci* **274**: 303–313.

Koch, H., and Schmid-Hempel, P. (2011a) Bacterial communities in central european bumblebees: low diversity and high specificity. *Microb Ecol* **62**: 121–133.

Koch, H., and Schmid-Hempel, P. (2011b) Socially transmitted gut microbiota protect bumble bees against an intestinal parasite. *Proc Natl Acad Sci USA* **108**: 19288–19,292.

Kosior, A., Celary, W., Olejniczak, P., Fijal, J., Król, W., Solarz, W., and Plonka, P. (2007) The decline of the bumble bees and cuckoo bees (Hymenoptera: Apidae: Bombini) of Western and Central Europe. *Oryx* **41**: 79–88.

Kwong, W.K., Engel, P., Koch, H., and Moran, N.A. (2014) Genomics and host specialization of honey bee and bumble bee gut symbionts. *Proc Natl Acad Sci U S A.* **111**: 11509–11,514.

Kwong, W.K., Mancenido, A.L., and Moran, N.A. (2017a) Immune system stimulation by the native gut microbiota of honey bees. *R Soc Open Sci.* **4**: 170003.

Kwong, W.K., Medina, L.A., Koch, H., Sing, K.-W., Soh, E.J. Y., Ascher, J.S., *et al.* (2017b) Dynamic microbiome evolution in social bees. *Sci Adv.* **3**: e1600513.

Ledgham, F., Quest, B., Vallaeys, T., Mergeay, M., and Covès, J. (2005) A probable link between the DedA protein and resistance to selenite. *Res Microbiol.* **156**: 367–374.

Lemly, A.D. (2004) Aquatic selenium pollution is a global environmental safety issue. *Ecotoxicol Environ Saf.* **59**: 44–56.

Lindblow-Kull, C., Kull, F.J., and Shrift, A. (1985) Single transporter for sulfate, selenate, and selenite in *Escherichia coli* K-12. *J Bacteriol.* **163**: 1267–1269.

Lloyd, J.R. (2003) Microbial reduction of metals and radionuclides. *FEMS Microbiol Rev.* **27**: 411–425.

Love, M.I., Huber, W., and Anders, S. (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* **15**: 550.

Maes, P., Rodrigues, P., Oliver, R., Mott, B.M., and Anderson, K.E. (2016) Diet related gut bacterial dysbiosis correlates with impaired development, increased mortality and Nosema disease in the honey bee *Apis mellifera*. *Mol Ecol.* **25**: 5439–5450.

Martinson, V.G., Danforth, B.N., Minckley, R.L., Rueppell, O., Tingek, S., and Moran, N.A. (2011) A simple and distinctive microbiota associated with honey bees and bumble bees. *Mol Ecol* **20**: 619–628.

McFrederick, Q.S., and Rehan, S.M. (2016) Characterization of pollen and bacterial community composition in brood provisions of a small carpenter bee. *Mol Ecol.* **25**: 2302–2311.

Meeus, I., De Graaf, D.C., Jans, K., and Smagghe, G. (2009) Multiplex PCR detection of slowly-evolving trypanosomatids and neogregarines in bumblebees using broad-range primers. *J Appl Microbiol.* **109**: no-no.

Meindl, G.A., and Ashman, T.-L. (2013) The effects of aluminum and nickel in nectar on the foraging behavior of bumblebees. *Environ Pollut.* **177**: 78–81.

Meindl, G.A., and Ashman, T.-L. (2014) Nickel accumulation by *Streptanthus polygaloides* (Brassicaceae) reduces floral visitation rate. *J Chem Ecol.* **40**: 128–135.

Mishra, R.R., Prajapati, S., Das, J., Dangar, T.K., Das, N., and Thatoi, H. (2011) Reduction of selenite to red elemental selenium by moderately halotolerant *Bacillus megaterium* strains isolated from Bhitarkanika mangrove soil and characterization of reduced product. *Chemosphere* **84**: 1231–1237.

Nadkarni, M.A., Martin, F.E., Jacques, N.A., and Hunter, N. (2002) Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set. *Microbiology* **148**: 257–266.

Näpflin, K., and Schmid-Hempel, P. (2016) Immune response and gut microbial community structure in bumblebees after microbiota transplants. *Proc R Soc B Biol Sci* **283**: 20160312.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., et al. (2017) vegan: Community Ecology Package.

Oremland, R.S., Herbel, M.J., Blum, J.S., Langley, S., Beveridge, T.J., Ajayan, P.M., *et al.* (2004) Structural and spectral features of selenium nanospheres produced by se-respiring bacteria. *Appl. Environ. Microbiol.* **70**: 52–60.

Overbeek, R., Begley, T., Butler, R.M., Choudhuri, J.V., Chuang, H.-Y., Cohoon, M., *et al.* (2005) The subsystems approach to genome annotation and its use in the project to annotate 1000 genomes. *Nucleic Acids Res.* **33**: 5691–5702.

Palmer-Young, E.C., Raffel, T.R., and McFrederick, Q.S. (2018) Temperature-mediated inhibition of a bumblebee parasite by an intestinal symbiont. *Proc R Soc B Biol Sci* **285**: 20182041.

Pennington, M.J., Rothman, J.A., Dudley, S.L., Jones, M.B., McFrederick, Q.S., Gan, J., and Trumble, J.T. (2017a) Contaminants of emerging concern affect *Trichoplusia ni* growth and development on artificial diets and a key host plant. *Proc Natl Acad Sci* **114**: E9923–E9931.

Pennington, M.J., Rothman, J.A., Jones, M.B., McFrederick, Q. S., Gan, J., and Trumble, J.T. (2017b) Effects of contaminants of emerging concern on *Megaselia scalaris* (Lowe, Diptera: Phoridae) and its microbial community. *Sci Rep* **7**: 8165.

Pennington, M.J., Rothman, J.A., Jones, M.B., McFrederick, Q.S., Gan, J., and Trumble, J.T. (2018) Effects of contaminants of emerging concern on *Myzus persicae* (Sulzer, Hemiptera: Aphididae) biology and on their host plant, *Capsicum annuum*. *Environ Monit Assess* **190**: 125.

Pinder, L.C.V. (1986) Biology of freshwater chironomidae. *Annu Rev Entornol* **31**: 1–23.

Powell, J.E., Martinson, V.G., Urban-Mead, K., and Moran, N.A. (2014) Routes of acquisition of the gut microbiota of the honey bee *Apis mellifera*. *Appl Environ Microbiol* **80**: 7378–7387.

Powell, J.E., Ratnayeke, N., and Moran, N.A. (2016) Strain diversity and host specificity in a specialized gut symbiont of honeybees and bumblebees. *Mol Ecol* **25**: 4461–4471.

Price, M.N., Dehal, P.S., and Arkin, A.P. (2010) FastTree 2-approximately maximum-likelihood trees for large alignments. *PLoS One* **5**: e9490.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., *et al.* (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* **41**: D590–D596.

Quinn, C.F., Prins, C.N., Freeman, J.L., Gross, A.M., Hantzis, L.J., Reynolds, R.J.B., *et al.* (2011) Selenium accumulation in flowers and its effects on pollination. *New Phytol.* **192**: 727–737.

R Core Team (2018) R: A language and environment for statistical computing.

Raymann, K., Motta, E.V.S., Girard, C., Riddington, I.M., Dinser, J.A., and Moran, N.A. (2018) Imidacloprid decreases honey bee survival rates but does not affect the gut microbiome. *Appl Environ Microbiol.* **84**: 00545–00518.

Rother, M., Wilting, R., Commans, S., and Böck, A. (2000) Identification and characterisation of the selenocysteine-specific translation factor SelB from the archaeon *Methanococcus jannaschii*. *J Mol Biol* **299**: 351–358.

Rothman, J.A., Carroll, M.J., Meikle, W.G., Anderson, K.E., and McFrederick, Q.S. (2018) Longitudinal effects of supplemental forage on the honey bee (*Apis mellifera*) microbiota and inter- and intra-colony variability. *Microb Ecol* **76**: 814–824.

Sadd, B.M., Barribeau, S.M., Bloch, G., de Graaf, D.C., Dearden, P., Elsik, C.G., *et al.* (2015) The genomes of two key bumblebee species with primitive eusocial organization. *Genome Biol.* **16**: 76.

Salter, S.J., Cox, M.J., Turek, E.M., Calus, S.T., Cookson, W.O., Moffatt, M.F., *et al.* (2014) Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. *BMC Biol.* **12**: 87.

Senderovich, Y., and Halpern, M. (2013) The protective role of endogenous bacterial communities in chironomid egg masses and larvae. *ISME J.* **7**: 2147–2158.

Soda, S., Kashiwa, M., Kagami, T., Kuroda, M., Yamashita, M., and Ike, M. (2011) Laboratory-scale bioreactors for soluble selenium removal from selenium refinery wastewater using anaerobic sludge. *Desalination* **279**: 433–438.

Sovik, E., Perry, C.J., LaMora, A., Barron, A.B., and Ben-Shahar, Y. (2015) Negative impact of manganese on honeybee foraging. *Biol Lett* **11**: 20140989.

Sura-de Jong, M., Reynolds, R.J.B., Richterova, K., Musilova, L., Staicu, L.C., Chocholata, I., *et al.* (2015) Selenium hyperaccumulators harbor a diverse endophytic bacterial community characterized by high selenium resistance and plant growth promoting properties. *Front Plant Sci.* **6**: 113.

Therneau, T.M. (2015a) A Package for Survival Analysis in S.

Therneau, T.M. (2015b) coxme: Mixed Effects Cox Models.

Velthuis, H.H.W., and van Doorn, A. (2006) A century of advances in bumblebee domestication and the economic and environmental aspects of its commercialization for pollination. *Apidologie* **37**: 421–451.

Veres, Z., and Stadtman, T.C. (1994) A purified selenophosphate-dependent enzyme from *Salmonella typhimurium* catalyzes the replacement of sulfur in 2-thiouridine residues in tRNAs with selenium. *Proc Natl Acad Sci* **91**: 8092–8096.

Veres, Z., Tsai, L., Scholz, T.D., Politino, M., Balaban, R.S., and Stadtman, T.C. (1992) Synthesis of 5-methylaminomethyl-2-selenouridine in tRNAs: 31P NMR studies show the labile selenium donor synthesized by the selD gene product contains selenium bonded to phosphorus. *Proc Natl Acad Sci* **89**: 2975–2979.

Vickerman, D.B., Trumble, J.T., George, G.N., Pickering, I.J., and Nichol, H. (2004) Selenium biotransformations in an insect ecosystem: effects of insects on phytoremediation. *Environ Sci Technol* **38**: 3581–3586.

Wang, Y., Shu, X., Zhou, Q., Fan, T., Wang, T., Chen, X., *et al.* (2018) Selenite reduction and the biogenesis of selenium nanoparticles by *Alcaligenes faecalis* se03 isolated from the gut of monochamus alternatus (Coleoptera: Cerambycidae). *Int J Mol Sci.* **19**: 2799. https://doi.org/10.3390/ijms19092799.

Zhang, W., Guo, R., Yang, Y., Ding, J., and Zhang, Y. (2016) Long-term effect of heavy-metal pollution on diversity of gastrointestinal microbial community of *Bufo raddei*. *Toxicol. Lett.* **258**: 192–197.

Zheng, H., Nishida, A., Kwong, W.K., Koch, H., Engel, P., Steele, M.I., and Moran, N.A. (2016) Metabolism of toxic sugars by strains of the bee gut symbiont *Gilliamella apicola*. *mBio* **7**: e01326–e01316.

Zheng, H., Powell, J.E., Steele, M.I., Dietrich, C., and Moran, N.A. (2017) Honeybee gut microbiota promotes host weight gain via bacterial metabolism and hormonal signaling. *Proc Natl Acad Sci USA* **114**: 4775–4780.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1: Survival plot the pilot project (Exp. 2) 0.75 mg/kg sodium selenate challenge on bumble bee workers that were inoculated with a microbiome versus uninoculated bees (N = 155, Z = −3.27, P = 0.001); (Cox mixed-effects model fitted with penalized log-likelihood: χ^2 = 54.34, d.f. = 2.7, P < 0.001, BIC = 40.70). Inoculated bees lived significantly longer than germ-free bees (average of 5.35 $\pm$ 0.44 days versus 3.77 $\pm$ 0.31 days respectively. Shaded areas represent 95% confidence intervals, and dashed lines indicate 50% survival probability.

Fig. S2: Boxplot of the results of our qPCR verification analysis showing that our technique rears bumble bees that are considered to be germ free. Uninoculated bees contained an average 16S rRNA gene copy number of 8.89×10^4 versus 6.88×10^8 in microbe-inoculated bees (Welch's two-tailed t-test, t = 3.13, P = 0.004). Error bars represent 1.5x the interquartile range of the data.

Fig. S3: Rarefaction analyses for each sample by observed ESVs.

Supplemental table ST1: Selenium metabolism genes identified using RAST in each of the bacteria relevant to this study. Numbers correspond to the frequency of each gene found in each bacterial genome. Full annotations can be found in Supplementary Data File SF1. '*' denotes taxa in which at least one ESV was differentially abundant in selenate treatments versus controls and '#' corresponds to bacteria that have been found in bumble bee guts.

Supplementary File SF1: Rapid Annotation using Subsystems Technology (RAST) tables for each of the relevant bacterial taxa.

Supplementary File SF2: Exact Sequence Variant (ESV) tables with ESV identifiers, SILVA taxonomy and the top BLAST hit for each ESV. Also contains the identifiers for the differentially abundant ESVs.